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Laboratory Assessment of Trace Element and Mineral Status^{1,2}

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1. INTRODUCTION

Few entirely satisfactory laboratory methods have been established for the clinical evaluation of the status of most trace elements or minerals in humans. Measurements of metalloenzyme activities have been proposed as useful assessment tests because plasma or serum trace metal concentrations are often affected by factors not related to the whole-body mineral element status. A simultaneous battery of tests involving body tissue or fluid elemental determinations, metalloenzyme assays, and functional-morphological indices provides the most reliable assessment of mineral element status. However, in a clinical diagnostic setting it is most practical to assess trace mineral status by analysis of a single blood specimen. The use of hair mineral content as an indicator of status is somewhat limited. Whereas low metal concentration in hair may be indicative of metal depletion, "normal" or high amounts do not necessarily preclude depletion or indicate toxic amounts (1), because of hair's susceptibility to environmental contamination and other problems. Further investigations are needed to establish the clinical value of whole blood, platelet, leukocyte, erythrocyte, saliva, skin, and fingernail analyses as indices of trace mineral nutriture.

1.1. Sample Collection and Testing

Accurate determination of trace elements in biological specimens requires special precautions and presents special analytical difficulties. Sampling procedures must be carefully considered because heterogeneity of trace element distributions in tissues is the rule rather than the exception. Analysis of apparently homogeneous samples such as blood, sweat, or saliva can be significantly affected by sampling and processing procedures. For example, hemolysis or microhemolysis of a sample can lead to erroneously high plasma or serum values for iron, zinc, and manganese because red blood

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cell concentrations are 10-fold or more greater than those in plasma for these elements (2). Zinc concentrations are 5 to 15 percent higher in serum than plasma because of the release of zinc from erythrocytes and platelets during clotting. Conversely, the choice of anticoagulant affects plasma values by osmotic influences on fluid shifts from blood cells.

1.2. Sample Contamination

The primary analytical problem encountered in trace element analysis is external contamination. Many trace elements are present in the laboratory environment in nanogram and even microgram amounts. Thus, a significant portion of an analytical value may be the result of contamination unless extraordinary measures are taken. This is a major reason for the wide variation of reported reference values, particularly for mineral elements present in the parts per billion range. A laboratory contemplating trace element analysis must be prepared to take precautions, to a point of fanaticism, for all sampling, preparation, and analytical procedures to assure that contamination is minimized.

Major sources of contamination in the laboratory include dust, rubber, paper products, wood, metal surfaces, skin, dandruff, and hair. Plastic and borosilicate glass are best suited for trace element analysis. Of these, fluorocarbon, polyethylene, and polypropylene plastics are generally the best. Prior to use, glassware should be cleaned of surface trace metal contamination by soaking overnight in dilute nitric acid or diluted commercial metal-scavenging solutions, followed by a thorough rinsing with deionized water. Water should meet, or exceed American Chemical Society specifications for type I water with greater than $14 \text{ M}\Omega/\text{cm}^2$ resistance. All anticoagulants and reagents used should be checked for trace element content prior to use. Only disposable plastic syringes with stainless steel needles should be used for blood collection. Stainless steel needles are not suitable for blood collection for chromium, nickel, and possibly other ultratrace metals unless they are siliconized, since stainless steel has a high chromium and nickel content. Evacuated blood collection tubes specified for trace metal analysis are also suitable if tops are removed after collection and the sample is not allowed to come in contact with the stopper. Leaching of metals from the stoppers of these and other types of tubes may contaminate the specimen.

1.3. ANALYTICAL METHODS

Any analytical method used for the determination of trace and ultratrace mineral elements in biological specimens must be sensitive, specific, precise, accurate, and relatively fast. Analytical sensitivity is extremely important because concentrations of trace or ultratrace elements in most biological samples are in the nanogram to microgram per gram range. The choice of analytical technique is dependent on the sample type and the element to be determined, because method requirements are not the same for all mineral elements or specimens.

The most popular techniques currently used for the determination of trace elements in biological specimens include photometry, atomic absorption spectrophotometry (AAS), and emission spectroscopy, which includes inductively coupled plasma emission spectroscopy (ICPES). Other techniques include inductively coupled plasma mass spectrometry, neutron activation analysis, X-ray fluorescence spectrometry, and electro-

chemical techniques. The most practical for use in most clinical settings are AAS and ICPEs techniques. ICPEs provides simultaneous multielement detection.

AAS is the method of choice for most routine trace mineral element analyses. Methods that involve diluted serum or plasma being aspirated directly into the AAS flame are effective for the determination of elements such as magnesium, zinc, and copper. Electrothermal, or flameless, AAS micromethods using as little as 10 μL of sample are used for many of the ultratrace elements or limited volume samples. Background correction using Zeeman or deuterium arc techniques is often necessary with electrothermal AAS methods to overcome matrix interferences. Matrix modifiers, reagents added to the serum or plasma sample to reduce background signals, may also be used. The sensitivities of the AAS techniques for determining the various elements depend upon the element of interest, sample, and on the technique used. The flame AAS technique is simpler and less tedious to perform than the flameless mode and is less subject to matrix interferences. Generally, if analyte concentrations of a specimen are below 50 ng/g, flameless techniques are necessary.

The ICPEs technique is a rapidly developing multielement method and is replacing even AAS as the method of choice for many trace element applications. ICPEs provides time savings because of simultaneous multielement determinations over a wide analytical range. Several useful reviews are available that discuss the principals of AAS and ICPEs and their applications to biological and clinical samples (2–5).

1.4 Quality Assurance

Effective quality control measures must be incorporated into trace analysis schemes because methods for trace element analysis are not standardized and are subject to matrix effects and contamination problems. An effective quality assurance program for trace or ultratrace element analyses requires the incorporation of the following into each batch of analyses:

1. reagent blanks,
2. replicate analyses to assess precision,
3. calibrators of the elements of interest in the expected concentration range of the specimens to be analyzed, and
4. a control or reference solution with known or certified concentrations of the trace elements to be determined to assess accuracy and batch to batch precision.

The reference material should be of the same matrix type and contain approximately the same amounts of analytes as the specimens. A wide variety of control or reference materials are available from several sources, for example, the National Institute of Standards and Technology. Recovery studies of elements from samples containing known quantities of added analyte are useful at regular intervals and when developing a method for assessing accuracy and linearity (Table 1).

2. CALCIUM AND PHOSPHOROUS

Calcium and phosphorous are the major mineral components of the body. These minerals occur in combination with organic and inorganic compounds and as free ions. Their two major roles are structural components of bone and regulatory agents in body

Table 1
Adult Reference Ranges for Selected Mineral Elements in Plasma (P) or Serum (S)

<i>Analyte</i>	<i>Reference Interval</i>	<i>Factors Affecting Concentration</i>
Total Calcium (S)	8.6–10.2 mg/dL	↑Hyperparathyroidism, alkaline antacids, some cancers, vitamin D toxicity ↓hypoparathyroidism, vitamin D deficiency, Mg deficiency, chronic renal failure.
Ionized Calcium (S)	4.64–5.28 mg/dL	
Chromium (S)	<0.05–0.5 µg/L	↓Diabetic children, pregnancy
Copper (S)	♀80–190 µg/dL ♂70–140 µg/dL	↑pregnancy, estrogen, birth control pills, infection, inflammation ↓Wilson's disease, Menkes' syndrome, protein malnutrition, cystic fibrosis.
Iron (S)	♀50–70 µg/dL ♂65–165 µg/dL	↑Hemochromatosis, acute leukemia, acute hepatitis, thalassemia, excessive Fe therapy. ↓Iron deficiency anemia, infection, hypothyroidism, kwashiorkor
Magnesium (S)	1.6–2.6 mg/dL	↑Dehydration, renal insufficiency, hypothyroidism. ↓Inadequate Mg intake/absorption, kwashiorkor, chronic alcoholism, hypercalcemia, pregnancy.
Manganese (S, P) (Whole Blood)	0.4–1.1 µg/L 7.7–12.1 µg/L	↑Industrial exposure, myocardial infarction, acute hepatitis. ↓Seizure disorders, phenylketonuria.
Molybdenum (S, P) (Whole Blood)	0.1–3.0 µg/L 0.8–3.3 µg/L	
Selenium (S, P) (Whole Blood)	7–160 µg/L 58–234 µg/L	↑Industrial toxicity. ↓Cardiomyopathy (Keshans Disease), GI cancer, pregnancy, cirrhosis, hepatitis.
Zinc (P)	70–150 µg/dL	↑Coronary heart disease, arteriosclerosis. ↓Estrogens, oral contraceptives, acute infections, acrodermatitis enteropathica, leukemias, pregnancy.

fluids. Bone also serves as a reservoir for these minerals. Calcium and phosphorous exist in the bones mostly as calcium hydroxyapatite and octacalcium phosphate.

2.1. Calcium

Calcium is the most abundant mineral in the body. More than 99 % of body calcium occurs in bone. The remainder, located in body tissues and extracellular fluids, is involved in several metabolic processes, such as blood coagulation, muscle contractibility, enzyme activation, nerve transmission, hormone function, and membrane transport. Calcium

exists in three physiochemical states in plasma. Approximately 50 % of plasma calcium is free or ionized, whereas about 40 % is bound to plasma proteins, chiefly albumin; its binding is pH dependent. Approximately 20 % of protein-bound calcium in serum is bound to the globulins. In some patients with multiple myeloma, the high concentrations of serum globulin may bind sufficient calcium to cause an increase in the total serum calcium concentration. The remaining 10 % of plasma calcium is complexed with small diffusible anions, including bicarbonate, lactate, citrate, and phosphate. Calcium can be redistributed among the three pools, acutely, or chronically, thus affecting the quantities of ionized and total calcium in the serum.

2.1.1. METHODS FOR ASSESSING CALCIUM STATUS

The method most commonly used for assessing calcium is either total or ionized (or free) calcium. Ionized calcium is considered to be more useful than total calcium as an indication of calcium status because it is biologically active and is tightly regulated by calcium-regulating hormones. Because calcium is bound to serum proteins, total calcium concentrations are greatly influenced by protein concentrations, especially albumin (6).

2.1.1.1. Total Serum Calcium. Although many methods have been used to determine total calcium in biological fluids, only three are in use today. These include photometric analysis, titration of a fluorescent calcium complex with EDTA or ethylene glycol tetraacetic acid (EGTA), or AAS. AAS has been approved by the National Committee for Clinical Laboratory Standards as the reference method for measuring serum concentrations of calcium (7). AAS provides better accuracy and precision than widely used spectrophotometric methods.

Total serum calcium is determined by AAS after diluting the specimen 1 to 50 with a solution of lanthanum-HCl (LaCl_3 , 10 mmol/L; HCl, 50 mmol/L). The lanthanum is used to prevent interference by phosphate. The diluted sample is aspirated into an air-acetylene flame. Detailed procedures for the determination of calcium in serum and reviews of this method have been published (8).

2.1.1.2. Serum Ionized Calcium Concentrations. Serum ionized calcium makes up approx 50 % of the calcium in serum and is the physiologically active form of calcium in the blood. Reductions in ionized calcium concentrations occur in hypoparathyroidism and vitamin D deficient rickets, and result in neuromuscular irritability. Elevated ionized calcium concentrations indicate functional hypercalcemia, and occur in patients with hyperparathyroidism or receiving chronic renal dialysis. Total serum calcium may be normal under these conditions.

Several factors affect the measurement of serum ionized calcium concentrations. These include changes in pH of the specimen, high concentrations of magnesium and sodium, and the presence of EDTA or heparin. Physiological anions such as citrate, phosphate, oxalate, and sulfate form complexes with free calcium and may lower its apparent concentration. Because most anticoagulants act by binding calcium, serum is the preferred specimen for the measurement of ionized, or free calcium. Standards that contain sodium and magnesium in the approximate concentration range of the sample will minimize the impact of these ions on the outcome. The binding of calcium by protein and small anions is affected by pH both in vivo and in vitro. Ideally, specimens should be analyzed at the pH of the patients blood because of the inverse relationship between pH and free, or ionized, calcium. Anaerobic conditions should be maintained because specimens lose carbon dioxide and become more alkaline on exposure to air. In addition, samples need

to be handled so as to minimize metabolism by red and white blood cells, which produce acids and reduce pH.

2.1.1.3. Calcium Reference Intervals. Serum calcium concentrations in healthy adults range from 8.6 to 10.2 mg/dL [2.15–2.55 mmol/L] (8). Concentrations decrease with age in men and females have slightly lower concentrations than males. Serum ionized calcium concentrations range between 4.64 to 5.28 mg/dL [1.16–1.32 mmol/L] in healthy adults. The reference interval for ionized calcium should be determined for a specific instrument, specimen type, and collection protocol. Ionized calcium values have been reported to vary between capillary blood and venous blood, and serum because of differences in pH. Correction to pH 7.4 eliminated the differences. Differences in reference intervals between laboratories are primarily a result of differences in sample handling and instrumentation.

2.2. *Phosphorous*

Phosphorous is the second most abundant mineral in the body. About 85 % of phosphorous in the adult body is present in the skeleton as either hydroxyapatite or as calcium phosphate. The remainder in cells and extracellular fluid is present as inorganic phosphate or in nucleic acids, phosphoproteins, phospholipids, and high energy compounds involved in cellular integrity and energy metabolism. Phosphorous is an essential factor in most of the energy-producing reactions of cells.

Phosphorous depletion results in low intracellular concentrations of phosphoglycerate and other energy-rich phosphate esters, failure of muscle contractility, impairment of oxygen delivery, severe muscular weakness, and cardiac and respiratory failure. Phosphorous depletion may occur in patients receiving long term total parenteral nutrition not supplemented with phosphorous, and in patients with keto-acidosis treated with insulin and unsupplemented with phosphorous. Suboptimal phosphorous status may also arise in people with prolonged and excessive intakes of antacids containing aluminum hydroxide or aluminum carbonate, because absorption of phosphate is impaired by high intakes of aluminum and other cations that form insoluble complexes with phosphate.

2.2.1. METHOD FOR ASSESSING PHOSPHOROUS STATUS

Serum phosphorous, measured as phosphate, is used most frequently to assess phosphorous status. Phosphate in serum exists both as the monovalent and divalent anion. The ratio of H_2PO_4^- : $\text{HPO}_4^{=}$ varies from 1:1 in acidosis to 1:4 at pH 7.4 and 1:9 in alkalosis. Approximately 55% of the phosphate in serum is free; 35% is complexed with sodium, calcium, and magnesium, and 10% is protein bound. Serum phosphorous concentrations are generally measured colorimetrically by a modification of the molybdenum blue method of Fiske and Subbarow (9). Serum is the specimen of choice for phosphorous determinations because many anticoagulants, such as citrate, oxylate, and EDTA interfere with formation of the phosphomolybdate complex. It is important to separate the cells from the serum as soon as possible because high concentrations of organic phosphate esters in the cells may be hydrolyzed to inorganic phosphate during storage.

2.2.2. PHOSPHOROUS REFERENCE INTERVALS

Reference intervals for serum phosphate concentrations vary considerably with age. Concentrations are about 50 % higher in infancy and decline throughout childhood until adult concentrations are reached. Serum phosphate, expressed as phosphorous, ranges from 2.5 to 4.5 mg/dL [0.81 to 1.45 mmol/L] in healthy adults, and 4.0 to 7.0 mg/dL [1.29 to 2.26 mmol/L] in children.

3. MAGNESIUM

Magnesium is the fourth most abundant cation in the body; within the cell it is second only to potassium. The adult human body (70 kg) contains 21 to 28 g of magnesium. Of this, about 60% is in bone, 20% in skeletal muscle, 19% in other cells, and about 1% in extracellular fluid.

There are two major roles for magnesium in biological systems: (1) it can compete with calcium for binding sites on proteins and membranes, and (2) it can form chelates with important intracellular ligands, notably adenosine triphosphate (ATP). Magnesium catalyzes or activates more than 300 enzymes in the body. Magnesium acts as an essential cofactor for enzymes concerned with cell respiration, glycolysis, and transmembrane transport of other cations.

The best defined manifestation of magnesium deficiency is impairment of neuromuscular function: examples are hyperirritability, tetany, convulsions, and electrocardiographic changes. Hypertension, preeclampsia, myocardial infarction, cardiac dysrhythmias, coronary vasospasm, and premature atherosclerosis have also been linked to magnesium depletion. Reviews that further detail the biochemical and clinical aspects of human magnesium nutrition are available (10–11).

3.1. Methods for Magnesium Assessment

Assessing magnesium status in humans is problematic because there is no simple, rapid, and accurate laboratory test to indicate total body magnesium status. For the past several decades, clinical chemistry laboratories have offered two tests to assess magnesium status: total serum magnesium concentration and urinary magnesium excretion. These two tests do not provide meaningful information about intracellular magnesium status, but only address the throughput of magnesium. Several other tests that may be of value in assessing magnesium status may be organized into three groups: tissue magnesium, ionized magnesium, and physiologic assessment of magnesium status.

3.1.1. SERUM OR PLASMA MAGNESIUM CONCENTRATION

Serum magnesium is the most frequently used index of magnesium status. However, the serum or plasma magnesium concentration provides only an approximate guide to the presence or absence of magnesium deficiency. Hypomagnesemia reliably indicates magnesium deficiency, but its absence does not exclude significant magnesium depletion. The concentration of magnesium in serum has not been shown to correlate with any other tissue pools of magnesium except interstitial fluid (12).

Serum is preferred rather than plasma because an additive such as an anticoagulant may be contaminated with magnesium or affect the assay procedure. Because the magnesium content of erythrocytes is three times as great as serum, it is important to prevent hemolysis and to harvest the serum promptly. Magnesium concentrations in serum are determined directly by flame AAS after diluting 50-fold with a lanthanum chloride or oxide diluent.

3.1.2. IONIZED MAGNESIUM CONCENTRATION

Magnesium in serum exists in several forms, protein bound (19–34% of total), as the free Mg^{2+} ion (61–67% of the total), and complexed to certain anions (5.5–14% of the total) (13). It is believed that free or ionized magnesium is the metabolically active form (14). The technology for measuring ionized magnesium in serum with ion specific electrodes has only recently been made available. Thus, more research is needed before the importance of

this test is fully realized. Ionized magnesium location and concentrations in tissues may be estimated by the use of fluorescent probes or by nuclear magnetic resonance spectroscopy. At present these techniques are limited to the research laboratory, but may have a future use in diagnosing disturbances in magnesium status and metabolism.

3.1.3. MAGNESIUM CONCENTRATION IN MUSCLE

Muscle contains approx 27% of the total body magnesium. Thus, it is an important tissue for magnesium status assessment. Needle biopsy has been used to determine the magnesium concentration in muscle, but this procedure is invasive, requires special skills, and the assay is tedious (12).

3.1.4. MAGNESIUM CONCENTRATION IN BLOOD CELLS

The mononucleated white cell (MNC) magnesium concentration has been proposed as a possible index of intracellular magnesium. In humans, magnesium concentrations in MNCs do not correlate with serum or erythrocyte concentrations. However, several studies show a correlation between the MNC magnesium concentration and muscle magnesium (15). The magnesium content of MNCs is reportedly a better indicator of cardiac arrhythmias associated with magnesium deficiency than is serum magnesium concentration (16).

As with serum, the erythrocyte magnesium concentration has not been shown to correlate significantly with other tissue pools of magnesium. Genetic regulation of this pool has been documented (17). Thus, the usefulness of determining erythrocyte magnesium in clinical medicine is unclear, even though changes in total erythrocyte magnesium have been linked to hypertension, premenstrual syndrome, and chronic fatigue syndrome (14).

3.1.5. MAGNESIUM RETENTION AFTER ACUTE ADMINISTRATION

Oral and intravenous magnesium loading tests have been described and are more widely used in clinical practice for diagnostic purposes than intracellular measurements. Normal individuals in magnesium balance excrete most (75–100%) injected magnesium in the urine within 24 to 48 h after administration, whereas individuals with a magnesium deficit retain a significant fraction of the injected magnesium. Patients who are to undergo this test should have normal kidney function, not be taking medication that affects kidney function and not have disturbances in cardiac conduction or advanced respiratory insufficiency.

3.2. Magnesium Reference Intervals

Total magnesium concentrations in healthy adults, as determined by AAS, range from 1.6–2.6 mg/dL (0.66–1.07 mmol/L) (7). Serum concentrations in newborns are slightly lower than in adults. Infants older than 5 mo, children, and adolescents have concentrations essentially the same as adults. Concentrations do not change appreciably throughout the day.

4. CHROMIUM

Chromium functions in the control of glucose and lipid metabolism. Studies have demonstrated that chromium is a potentiator of insulin action. Insulin resistance may be a consequence of chromium deficiency; insulin apparently is ineffective as a glucose regulator without chromium. Few definitive studies of human chromium deficiency have been carried out mainly because of analytical difficulties in determining ultratrace amounts of chromium in tissue. Evidence of human chromium deficiency is mostly

indirect, based on the improvement of insulin-resistant glucose tolerance after supplementation with chromium-containing compounds. Reviews that detail the biochemical and clinical aspects of chromium nutrition are available (18,19).

4.1. Methods for Chromium Assessment

No laboratory tests that reliably define body chromium status have been established as the determination of chromium in human tissues and fluids is one of the most difficult of trace metal determinations. Reported serum and urine chromium concentrations have been grossly overestimated in the past. As method sensitivity has improved, the capability of identifying and eliminating exogenous chromium contamination has resulted in substantially decreased estimates of chromium concentrations in biological specimens.

AAS with a graphite furnace and Zeeman background correction is the preferred method for estimating chromium in biological material (20). Strict avoidance of even brief contact with any metal surface by the sample must be avoided. Even stainless steel needles used for blood collection should be avoided unless they are siliconized.

4.2. Chromium Reference Intervals

The present accepted adult reference range for the chromium concentration in serum is <0.05 – $0.5 \mu\text{g/L}$ [1 – 10 nmol/L]. The presently established urinary excretion of chromium is between 100 and 200 ng/24 h. This may vary, depending upon recent chromium intake or the use of supplements.

5. COPPER

Copper is an integral component of many metalloenzymes, including ceruloplasmin, superoxide dismutase, dopamine- β -hydroxylase, ascorbate oxidase, lysyl oxidase, and tyrosinase. The major functions of copper metalloproteins involve oxidation-reduction reactions. Most known copper-containing enzymes bind and react directly with molecular oxygen.

A number of pathological conditions have been attributed to the loss of cuproenzyme activity. Failure of pigmentation has been attributed to the depressed tyrosinase activity required in the first step in the biosynthesis of melanin. A variety of connective tissue cross-linking defects (cardiac, vascular, and skeletal) are believed to be caused by a loss of amine oxidase activity, particularly that of lysyl oxidase. Ataxia may result from depressed cytochrome c oxidase activity in motor neurons. Depressed dopamine- β -hydroxylase activity may result in abnormal catecholamine conversions.

Reviews that discuss in detail the biochemical and clinical aspects of copper are available in this volume and elsewhere (20–24).

5.1. Methods for Copper Assessment

The assessment of copper nutriture in adult humans has not been perfected (25). However, there are a number of indices that are useful in the diagnosis of human copper deficiency.

5.1.1. SERUM OR PLASMA COPPER CONCENTRATIONS

Serum or plasma copper provides a relatively routine test for the clinical assessment of copper nutriture; low concentrations may be indicative of severely depleted copper stores. However, plasma copper concentrations are a poor indicator of short-term marginal copper status in humans. Plasma copper concentrations are regulated by strong homeostatic mechanisms and are

maintained within a relatively narrow range within an individual; plasma copper falls only after stores are severely depleted (26). Circulating copper concentrations are sensitive to factors which may not be directly related to copper nutriture. Women generally have higher plasma or serum copper concentrations than men, and estrogen increases plasma copper concentrations in both younger women taking oral contraceptives and postmenopausal women on estrogen therapy (27). Other conditions that result in increased plasma copper concentrations include pregnancy, infections, inflammation, and rheumatoid arthritis (28). Increased serum copper has also been reported in patients with dilated cardiomyopathy and immediately following myocardial infarction (29–30). Conversely, corticosteroid and corticotropin tend to decrease plasma copper concentrations (28). Thus, conditions that elevate serum copper may obscure changes in copper status, even during copper deprivation. Conditions that decrease plasma copper also need to be ruled out before a proper assessment may be made.

AAS after direct dilution with deionized water is the method of choice for determining serum or plasma copper concentrations. Hemolysis is not a great concern for copper determinations because concentrations of copper in plasma and erythrocytes are nearly equal.

5.1.2. CERULOPLASMIN

Most of the changes observed in plasma copper concentrations are associated with changes in the cuproprotein, ceruloplasmin (EC 1.16.3.1); over 70–80% of the plasma copper is associated with ceruloplasmin. Both the enzyme activity of ceruloplasmin and the immunoreactive ceruloplasmin protein respond in a similar manner to age, gender, and hormone use. They are also both elevated in pregnancy, and as a result of inflammatory responses. Enzymatically measured ceruloplasmin has been shown to be a sensitive indicator of copper status in several species of animals and has been found to be depressed in some men and women during copper deprivation studies (31).

Serum ceruloplasmin may be measured immunochemically or by its oxidase activity. It is likely that a copper-depleted apoceruloplasmin is present in normal and copper-deficient serum (32). Thus, assays of its oxidase activity may be preferred to immunological methods. Recent studies of experimental copper deprivation demonstrated that the specific activity of ceruloplasmin, defined as the ratio of the enzyme activity to the immunoreactive protein, is a better indicator of copper status than either the enzyme activity or immunoreactive protein alone (31). Ceruloplasmin specific activity is sensitive to copper status and is inversely related to the autonomic blood pressure response in young women (33). It is not affected by age, gender, or hormone use (27).

5.1.3. SUPEROXIDE DISMUTASE ACTIVITY

Erythrocyte copper-zinc superoxide dismutase (EC 1.15.1.1) activity is depressed during copper deficiency in several animal species and in humans. It has also been shown to be sensitive to changes in copper status in several studies of experimental copper deprivation (31). In contrast to plasma copper or ceruloplasmin, erythrocyte copper-zinc superoxide dismutase activity does not seem to be affected by age, gender, or hormone use (27). Recent studies, however, have suggested that some conditions that produce oxidative stress may increase copper-zinc superoxide dismutase activity, even during periods of low copper intake; erythrocyte superoxide dismutase activity was elevated in competitive swimmers during training, presumably as a functional adaptation to increased oxygen utilization during aerobic training (34).

Most available assays are based on the indirect measurement of activity that consists of a superoxide generating system and a superoxide indicator that is measured spectrophotometrically (35). Addition of copper-zinc superoxide dismutase inhibits the absorption change. However many of these assays are prone to interferences. A method based on the autoxidation of pyrogallol (36) is probably the method of choice and seems to be relatively free of interferences.

A major disadvantage related to the measurement of superoxide dismutase is the lack of a standard assay. In spite of the plethora of methods that provide apparently different numbers and definitions of "units," analysis of copper-zinc superoxide dismutase in erythrocytes can provide clinically useful data if reference ranges are established in each laboratory and conditions used for analysis are carefully maintained.

5.1.4. CYTOCHROME C OXIDASE ACTIVITY

Depressed tissue cytochrome c oxidase (EC 1.9.3.1) activity is an early and consistent sign of copper deficiency in animals. Defects in cytochrome c oxidase activity can cause neurological, cardiac, and muscle defects when the activity is only about 50% of normal (37). Markedly lower leukocyte cytochrome c oxidase activity has also been reported in patients with Menkes disease (38) (*see* Chapter 12). Platelet and leukocyte cytochrome c oxidase activity was reduced in young women on a low copper intake (32). Platelet cytochrome c oxidase activity was the most sensitive indicator of changes in copper status in a recent study with postmenopausal women (39). Studies with rats demonstrated that both platelet and leukocyte cytochrome c oxidase activity is sensitive to copper status (40,41); the cytochrome c oxidase activity in platelets correlates with liver copper, an established marker of copper status in animals.

Most methods for determining cytochrome c oxidase activity in tissues and blood cells are based on the spectrophotometric analysis of the oxidation of ferricytochrome c (20). A microassay has been described that utilizes a coupled reaction between cytochrome c and 3,3'-diaminobenzidine tetrachloride in microwell plates (42).

Platelet and mononuclear leukocyte cytochrome c oxidase activities are higher in older adults than in young adults, but are not affected by gender or hormone use (27). However, there seems to be large subject to subject variation, the enzyme is fairly labile, and cytochrome c oxidase assays are sensitive to minor variations in technique.

5.1.5. OTHER POTENTIAL COPPER INDICATORS

Other potential indices of copper status, which have not been well investigated or are too tedious for routine use, include platelet, erythrocyte or leukocyte copper content, skin lysyl oxidase activity, and measurements of copper retention and turnover.

5.2. Copper Reference Intervals

Serum copper concentrations are higher in women of child bearing age, 80 to 190 $\mu\text{g/dL}$ [12.6 to 24.4 $\mu\text{mol/L}$] than in men, 70 to 140 $\mu\text{g/dL}$ [11 to 22.0 $\mu\text{mol/L}$]; serum copper is highest in pregnant women, 118 to 302 $\mu\text{g/dL}$ [18.5 to 47.4 $\mu\text{mol/L}$]. The reference interval for infants is 20 to 70 $\mu\text{g/dL}$ [3.1 to 11.0 $\mu\text{mol/L}$] and 80 to 190 $\mu\text{g/dL}$ [12.6 to 29.9 $\mu\text{mol/L}$] in children 6 to 12 yr of age (7).

6. IRON

Iron deficiency is the most prevalent micronutrient deficiency in both industrialized and developing countries (*see* Chapter 6). It is particularly common in children and pregnant

women. Severe iron deficiency results in anemia, which may be accompanied by loss of energy, anorexia, increased susceptibility to infection, abnormalities in thermogenesis and behavior, and reductions in intellectual performance and work capacity. Iron overload is also a problem that occurs most frequently with idiopathic hemochromatosis, a potentially fatal hereditary disease characterized by a progressive accumulation of iron in tissues, and may contribute to heart disease (*see* Chapter 12). Iron overload also may result from excessive intakes of dietary or medicinal iron, injections of therapeutic iron, or blood transfusions.

Iron functions mainly in the transport of oxygen from the environment to the terminal oxidases. Iron is also involved in electron transport and in oxidation-reduction reactions in the body. Reviews that detail the biochemical and clinical aspects of iron nutrition are available (43–45).

6.1. Methods for Iron Assessment

The iron status of humans can range from dangerous iron overload to severe iron-deficiency anemia. Thus, many different methods have been used to assess the iron status of an individual. These include hemoglobin, hematocrit, mean cellular hemoglobin, mean cell volume, free erythrocyte protoporphyrin, bone marrow iron stain, serum iron, total iron binding capacity, serum transferrin, transferrin saturation, serum ferritin, and more recently, serum transferrin receptors. These methods vary in their specificity and sensitivity.

6.1.1. HEMOGLOBIN AND HEMATOCRIT

Measurement of hemoglobin concentration in whole blood probably is the most widely used screening test for iron deficiency anemia. However, it is relatively insensitive and has low specificity. Concentrations fall only in the third stage of iron deficiency, and considerable overlap exists between iron deficient and normal nonanemic individuals. Hemoglobin concentrations also are altered by several conditions, such as dehydration, cigarette smoking, chronic inflammation, infection, protein energy malnutrition, pregnancy, folic acid deficiency, and vitamin B₁₂ deficiency. Hemoglobin can be measured spectrophotometrically in whole blood, anticoagulated with EDTA or heparin, and after conversion to cyanmethemoglobin.

The hematocrit falls after hemoglobin production has become impaired. It is also relatively insensitive and nonspecific because hematocrit is affected by the same factors that affect hemoglobin, including changes in plasma volume.

6.1.2. FERRITIN

There is a close correlation between serum ferritin concentration and storage iron (46). In a controlled study of young women depleted of iron by a diet low in iron and phlebotomy, then repleted with iron, serum ferritin was the most sensitive measured indicator of changes in iron status and stores (47). Concentrations of less than 12 µg/L are believed to indicate depletion of body stores, while concentrations above 300 µg/L indicate iron overload. Elevated serum ferritin concentrations also occur with both acute and chronic inflammations, vitamin B₁₂ and folic acid deficiencies, liver disease, leukemia, hyperthyroidism, and Hodgkin's Disease (46). Serum ferritin concentrations are determined by immunological techniques. A number of commercial kits are available.

6.1.3. SERUM IRON, TOTAL IRON-BINDING CAPACITY, TRANSFERRIN, AND TRANSFERRIN SATURATION

Serum iron and total iron binding capacity reflect the iron in transit from the reticuloendothelial system to the bone marrow. Transferrin, the serum transport protein, is about

one-third saturated with iron in normal circumstances. Transferrin may be measured immunologically, but is often determined as total iron binding capacity. The most useful measure of iron transport is transferrin saturation, the ratio of serum iron and total iron binding capacity, because plasma iron and total iron binding capacity move in reciprocal fashion in both iron deficiency and overload. A transferrin saturation below 16 % is indicative of an under supply of iron to the body, whereas saturation of over 55 % is diagnostic of iron overload or hemochromatosis.

Serum or plasma iron may be measured by using chromogens such as ferrozine or bathophenanthroline sulphonate. Total iron binding capacity is determined by saturating the serum with excess iron, followed by the addition of magnesium carbonate, which removes all of the iron not bound to transferrin. Determination of serum iron by AAS is not advisable because, unlike the colorimetric procedure, AAS will also measure the heme iron released from hemolysed erythrocytes.

6.1.4 TRANSFERRIN RECEPTOR

Transferrin receptors are transmembrane proteins present on the surface of most cells. Studies have shown that serum concentrations of transferrin receptors increase in iron-deficiency anemia, thus making it a useful marker in diagnosing microcytic anemias (48). Circulating transferrin receptor concentrations increase in tissue iron deficiency; this reflects the degree of iron deficiency in the erythroid precursors in the marrow. In a controlled study (49), serum transferrin receptors declined as iron stores were depleted. Circulating transferrin receptor concentrations increased only after iron stores were depleted, but before changes in other markers of iron deficiency, such as transferrin saturation, mean red cell volume, and erythrocyte protoporphyrin concentrations. The ratio of transferrin receptor to ferritin displays an inverse relationship to iron status, covering the spectrum from usual iron stores in health to substantial iron deficiency (49). Thus, this ratio may be a useful indicator of preanemic iron status. Unlike ferritin, transferrin receptor concentrations are not significantly affected by inflammation or by liver disease (50). Transferrin receptor concentrations can be determined by enzyme immunoassay systems that are now available.

6.1.5. FREE ERYTHROCYTE PROTOPORPHYRIN AND ZINC PROTOPORPHYRIN

Free erythrocyte protoporphyrin and zinc protoporphyrin concentrations have been shown to be sensitive indices of iron-deficient erythropoiesis. In iron deficiency, porphyrins accumulate in the erythrocytes because the lack of iron decreases the rate of heme synthesis (51). Changes in free erythrocyte protoporphyrin or zinc protoporphyrin are relatively insensitive to acute changes in iron status because iron stores must be depleted before heme synthesis is affected, and because of the relatively slow turnover of erythrocytes (47). Porphyrins also increase in lead poisoning because lead interferes with several enzymes involved with heme synthesis. Free erythrocyte protoporphyrin and zinc protoporphyrin concentrations are measured spectrofluorometrically (44).

6.2. Iron Reference Intervals

Serum iron concentrations range from 65 to 165 $\mu\text{g/dL}$ [11.6 to 31.3 $\mu\text{mol/L}$] in men and 50 to 170 $\mu\text{g/dL}$ [9.0 to 30.4 $\mu\text{mol/L}$] in women (7). Total serum iron binding capacity in healthy adults ranges between 250 and 425 $\mu\text{g/dL}$ [44.8 and 76.1 $\mu\text{mol/L}$]. Serum ferritin concentrations range from 20 to 250 $\mu\text{g/L}$ in men and 10 to 120 $\mu\text{g/L}$ in

women. Ferritin concentrations below 10 $\mu\text{g/L}$ are indicative of depleted iron stores, whereas concentrations above 300 $\mu\text{g/L}$ indicate iron overload.

7. MOLYBDENUM

The essentiality of molybdenum for animals and humans is based on molybdenum being an essential component of three metalloenzymes: xanthine oxidase, aldehyde oxidase, and sulfite oxidase. Xanthine oxidase participates in the degradation of purines into uric acid. Aldehyde oxidase catalyzes the oxidation of aldehydes, and sulfite oxidase catalyzes the final stage of sulfur amino acid oxidation.

No well-defined cases of dietary human molybdenum deficiency have been reported. However, a single case was reported (53) of a patient on prolonged parenteral nutrition with a syndrome characterized by hypermethionemia, hypouricemia, hyperoxypurinemia, hypouricosuria, and low urinary sulfate excretion that was corrected by molybdenum. A possible congenital defect in molybdenum metabolism was suggested for an infant who showed feeding difficulties, mental retardation, skull asymmetry, and biochemical defects in xanthine and sulfite oxidase activities (52). Reviews that detail the biochemical and clinical aspects of molybdenum nutrition are available (53,54).

7.1. *Methods for Molybdenum Assessment*

Current methods for determining molybdenum in biological specimens are inadequate. The methods have mostly employed emission spectroscopy, neutron activation, and AAS techniques. Use of a nitrous oxide-acetylene flame has been suggested for molybdenum determinations by flame AAS. However, concentrations in biological specimens are so low that preconcentration or prior extraction is necessary prior to analysis.

7.2. *Molybdenum Reference Intervals*

Reported reference intervals for molybdenum in healthy adults range from 0.8 to 3.3 $\mu\text{g/L}$ [8.3 to 34.4 nmol./L] for whole blood, 0.1 to 3.0 $\mu\text{g/L}$ [1.0 to 34.4 nmol./L] for serum or plasma, and 8 to 34 $\mu\text{g/L}$ [83 to 354 nmol./L] for urine excretion.

8. MANGANESE

Manganese is associated with the formation of connective and bony tissue, with growth and reproductive functions and with carbohydrate and lipid metabolism. Important manganese-containing enzymes include arginase, pyruvate carboxylase, and manganese superoxide dismutase in mitochondria. Manganese can also act as an enzyme activator by binding to a substrate (such as ATP) or directly to the protein, causing conformational changes. Enzymes that are specifically activated by manganese include glycosyltransferases, glutamine synthetase, and phosphoenolpyruvate carboxykinase.

Manganese is accepted as essential for humans mainly on the basis of its proven role in manganese-dependent enzymes and on the production of manganese deficiency in experimental animals rather than on direct evidence of human deficiency. Although manganese deficiency has not been documented in humans consuming natural diets, some disease states have been linked to possible disturbances in manganese metabolism. Low blood and tissue manganese concentrations have been reported in children with seizure disorders without head trauma and in children with maple syrup disease and phenylketonuria (55). Additionally, manganese deficiency was suggested as an

underlying factor in the development of hip abnormalities, joint disease, and congenital malformations (56). Serum manganese concentrations are increased following industrial exposure, acute hepatitis and other liver diseases, and myocardial infarction. Increased erythrocyte concentrations have been reported in patients with rheumatoid arthritis. Manganese toxicity is a problem in manganese miners in Chile and other countries and in liver disease patients (*see* Chapter 17), due to the prominent role of biliary excretion in manganese homeostasis. Reviews that detail the biochemical and clinical aspects of manganese nutrition are available (55,56).

8.1. Methods for Manganese Assessment

Laboratory tests that reliably assess body manganese status have not been established. The most common method for estimating changes in manganese metabolism and status is to measure its concentration in whole blood or serum. It is likely that whole-blood manganese, or manganese in blood cells, may best reflect manganese stores in tissue. Recent evidence indicates that the manganese concentration and manganese superoxide dismutase activity in lymphocytes are sensitive to changes in manganese status (57,58). Widely varying concentrations for manganese in blood have been reported, up to a 35-fold range for serum. This large variation in reported manganese concentrations can be partly attributed to sample contamination during collection or processing, and the use of older, relatively insensitive and nonspecific analytical methods.

Because of the low concentrations of manganese in blood, hair, and urine, AAS with Zeeman background correction is the method of choice for manganese determinations. Instrumental parameters, pitfalls, and references to published methods for the flameless AAS determination of manganese have been reviewed (59,60).

8.2. Manganese Reference Intervals

Current acceptable adult reference intervals for blood manganese are 0.4 to 1.1 $\mu\text{g/L}$ [7.0 to 20.0 nmol./L] for serum or plasma and 7.7 to 12.1 $\mu\text{g/L}$ [140 to 220 nmol./L] for whole blood (59,60).

9. SELENIUM

Selenium helps defend the body against oxidant stress and is involved in the metabolism of thyroid hormones (*see* Chapter 13). The existence of a number of selenoproteins has been demonstrated. However, four different glutathione peroxidases and three different iodothyronine deiodinases are the only characterized selenoproteins with clearly defined functions. Selenoprotein P, isolated from plasma, may transport selenium between tissues and is an extracellular antioxidant defense protein. Selenoprotein W, found in muscle, may be involved in the pathogenesis of muscular degeneration seen in combined selenium and vitamin E deficiencies. All of the above proteins and enzymes are significantly decreased in selenium deficiency.

Selenium deficiency has been associated with two diseases of childhood in China. Keshan disease is an endemic cardiomyopathy that affects primarily children and women of child bearing age in areas of China with low soil selenium concentrations. Kashin-Beck disease, an endemic osteoarthritis that occurs during adolescent and preadolescent years, is another disease linked to low selenium status in China. In both instances, most indicators of selenium status are 30 to 40% lower than controls. Selenium supplementation has been

shown to prevent or control these diseases. Other factors, in addition to selenium, may also be involved in the etiology of these diseases. Patients who were intravenously fed preparations not supplemented with selenium exhibited low selenium status, and some developed cardiomyopathy and skeletal muscle weakness. The relation of selenium to human health and disease has been reviewed (61–63).

9.1. Methods for Selenium Assessment

The determinations of urinary and blood selenium are useful measures of human selenium status. Plasma or serum concentrations may be a more sensitive indicator of selenium status than whole blood concentrations. In China, hair selenium concentrations were found to correlate with blood concentrations of selenium and were used to assess risk of selenium deficiency. Assay of erythrocyte glutathione peroxidase activity has been shown to correlate with blood selenium and is useful as a functional test of selenium status. The determination of selenoprotein P, the major selenium-containing protein in plasma, is also a useful and sensitive test for selenium nutritional status.

Recommended methods for determining selenium in biological specimens are flameless AAS, with either deuterium arc (64) or Zeeman effect background correction (65), and spectrofluorometry. The capability of selenium in forming covalent organo-compounds impacts on its analytical determination in two ways: (1) the organo-selenium forms are likely to be quite volatile and therefore can be lost in certain sample preparation steps such as high temperature ashing; and (2) the facile reduction of sample selenium to the volatile hydride form allows the determination of selenium by the AAS hydride generation technique.

9.2. Selenium Reference Intervals

Reported selenium concentrations from healthy adults range from 58 to 234 $\mu\text{g/L}$ in whole blood, 75–240 $\mu\text{g/L}$ in erythrocytes, 46 to 143 $\mu\text{g/L}$ in serum or plasma, 7 to 160 $\mu\text{g/L}$ excretion in urine, and 0.2 to 1.4 $\mu\text{g/g}$ in hair (66). Blood and tissue selenium contents vary with the selenium status of the particular geographic area.

10. ZINC

The essentiality of zinc for growth and well-being of both plants and animals is well established. The metabolic functions of zinc are based largely on its presence in over 300 metalloenzymes involved in virtually all aspects of metabolism. Important zinc-containing enzymes in humans include carbonic anhydrase, alkaline phosphatase, RNA and DNA polymerases, thymidine kinase, carboxypeptidases, and alcohol dehydrogenase. Zinc also plays a major role in protein synthesis and has an important function in gene expression; the involvement in gene expression is both a structural role and an enzymatic role. For detailed information on the metabolic interactions of zinc refer to recent reviews (67–69).

Nutritional zinc deficiency in humans is fairly prevalent throughout the world; it was first documented in 1961 in Egyptian and Iranian males as being the result of a low zinc diet in which a high fiber content decreased the availability of zinc for intestinal absorption. As zinc deficiency progresses, the clinical manifestations exist as a spectrum. In mild zinc deficiency, oligospermia, weight loss, hyperammonemia and lowered alcohol tolerance have been observed (67). Moderate zinc deficiency is characterized by growth retardation in adolescents and children, hypogonadism in adolescent males, mild dermatitis, poor appetite, delayed wound healing, mental lethargy,

impaired immune responses, and abnormal dark adaptation. Manifestations of severe zinc deficiency, as in the disease acrodermatitis enteropathica, include bullous-pustular dermatitis, alopecia, diarrhea, weight loss, recurrent infection, neuropsychiatric disorders, and ultimately death if not treated.

10.1. Methods for Zinc Assessment

Laboratory tests for assessing zinc status can be classified into two groups: those involving analysis of zinc in a body tissue or fluid and those testing a zinc-dependent function. Useful tests in the first category include determinations of the zinc content of the plasma or serum, blood cells, urine, and saliva. Functional tests include measurements of activities of zinc-containing enzymes and assessment of taste acuity. Other tests that are either too complex for routine diagnosis or not well investigated include measurement of changes of plasma zinc during exercise, blood ethanol clearance, zinc balance, ^{65}Zn uptake by erythrocytes, and ^{65}Zn retention and turnover.

Although the above tests have been shown to be related to zinc depletion in humans and animals, no single test has been proven to be a definitive indicator of zinc status (20). Test results must be interpreted with caution because they may be confounded by clinical conditions unrelated to the subjects' zinc status *per se*.

10.1.1. PLASMA OR SERUM ZINC

Although the zinc concentration in plasma or serum has often been shown to indicate human zinc deficiency, it does not reflect whole-body zinc status in all cases. Other conditions that depress plasma zinc without causing deficiency include nonfasting states, infection, inflammation, administration of steroids, pregnancy, and hypoalbuminemic conditions, such as hepatic cirrhosis and malnutrition.

The determination of plasma or serum zinc concentrations by AAS is the simplest and, analytically, most reliable test for the routine assessment of zinc nutriture. An AAS method using a fivefold diluted plasma, and standards in 5% glycerol matrix are recommended for plasma or serum zinc determination (70). Hemolysis must be avoided during sample acquisition and preparation because erythrocytes contain at least ten times more zinc than plasma.

10.1.2. HAIR ZINC CONCENTRATION

Low hair zinc concentrations have been documented in zinc-deficient, Egyptian dwarfs, in zinc deficient US infants and children, and in conditions associated with zinc deficiency, such as sickle cell anemia, acrodermatitis enteropathica, and celiac disease. However, in some cases of severe zinc deficiency, above normal hair zinc concentrations were attributed to zinc accumulation in hair whose growth rate was decreased as a result of the deficiency. Environmental contamination can also lead to apparently high hair zinc concentrations. Correlations between hair zinc and blood or tissue zinc are usually poor. Thus hair zinc is unreliable as a measure of zinc status.

10.1.3. URINARY ZINC EXCRETION

Decreased urinary zinc excretion usually accompanies human zinc deficiency. However, conditions associated with zinc depletion, such as hepatic cirrhosis, high alcohol intake, sickle cell anemia, total parenteral nutrition, and postsurgical periods, often result in increased urinary zinc excretion.

10.1.4. ZINC IN LEUKOCYTES

Studies have indicated reductions in the apparent zinc content of peripheral leukocytes in experimental zinc depletion and other conditions related to zinc deficiency (71). However, other investigators using different cell separation techniques were unable to confirm these findings (72). The apparent changes in zinc content of the leukocyte fractions apparently were related to the degree of contamination by blood platelets.

10.1.5. ZINC-CONTAINING ENZYME ACTIVITIES

Several zinc-dependent enzymes such as alkaline phosphatase, carbonic anhydrase, nucleoside phosphorylase, and ribonuclease are useful indicators of zinc deficiency. Depression of alkaline phosphatase activity in either serum or neutrophils has been observed in a number of human zinc-deficient conditions. A study of zinc-deficient patients supplemented with zinc showed increases in alkaline phosphatase activity that paralleled the degree of zinc repletion. However, as with serum zinc, alkaline phosphatase activities are nonspecific and are affected by conditions unrelated to zinc status. In patients with sickle cell anemia whose zinc nutriture was impaired, carbonic anhydrase and nucleoside phosphorylase activities were related to zinc status and responded to zinc supplementation (71). Zinc metalloenzyme assay methods that require zinc in the reagents are not suitable tests for zinc status.

10.1.6. OTHER POTENTIAL ZINC INDICES

Metallothionein I concentration in plasma or erythrocytes was proposed as a potentially useful index of zinc status; it was related to zinc status in some human and animal studies (73). However, questions have been raised about the effects of other minerals on this measurement (74). Recent studies have also suggested that serum extracellular superoxide dismutase, thymulin, and plasma 5' nucleotidase may also be sensitive to zinc deprivation. However, more rigorous evaluation is needed before these can be accepted as specific indicators of zinc status.

10.2. Zinc Reference Intervals

The accepted reference interval for zinc in plasma is 70 to 150 $\mu\text{g/dL}$ [10.7 to 22.9 $\mu\text{mol/L}$]. Serum zinc concentrations are generally 5 to 15 % higher than plasma because of osmotic fluid shifts from blood cells when various anticoagulants are used. A fasting morning sampling is important for zinc determination because plasma zinc exhibits both circadian and postprandial fluctuations.

11. OTHER ELEMENTS

Arsenic, boron, nickel, vanadium, and silicon are considered possibly essential trace or ultratrace elements with tissue concentrations in the nanogram per gram amounts. These elements are most likely essential to animals and thus may be essential to humans. However, no well-defined cases of deficiency of these elements have been described in humans. Several publications review the evidence for essentiality and proposed functions of these elements (54,75) including Chapter 2 of this book.

11.1. Methods for Assessment

Currently there are no well defined methods for evaluating the nutritional status of these elements in humans. Several methods have been described for measuring these elements in biological specimens.

11.1.1. ARSENIC

Techniques that have been commonly used to determine arsenic in biological specimens are mass spectrometry, neutron activation analysis, emission spectroscopy, and AAS. For most laboratories, AAS may be the most practical technique. Hydride generation AAS methods are required for arsenic determination in most situations because organo-arsenic forms may be quite volatile.

11.1.2. BORON

The boron content of human tissues and body fluids is poorly documented mainly because of contamination and methodological problems associated with earlier methods of analysis. A technique involving low-temperature wet ashing of specimens in Teflon containers and measurement of boron by ICPEs shows promise for use in the clinical setting (76). Use of borosilicate glass apparatus should be avoided.

11.1.3 NICKEL

Sensitive techniques are required for the determination of the ultratrace amounts of nickel in biological specimens. Flameless AAS is recommended. Instrumental parameters and published methods for AAS determination of nickel in biological samples have been summarized (3). Contact of specimens with stainless steel must be avoided.

11.1.4 VANADIUM

Current analytical techniques for vanadium in biological specimens are inadequate. The techniques most commonly employed are neutron activation analysis and flameless AAS (54).

11.1.5. SILICON

The techniques that have been used for determining silicon concentrations in biological materials include mass spectrometry, ICPEs, and AAS (54).

11.2. Reference Intervals

Because of the low concentrations and tentative nature of many of the analytical techniques, there are no reliable or established reference ranges for these elements in human fluids and tissues except for toxic levels of arsenic. Compilations of reported concentrations and comments on the reliability of the data are found in several references (77,78).

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